DISCUSSION

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Control of body weight and energy balance is a complex process in which there is a link between fat tissue, gut, pancreas, and brain. There is a link between certain brain nuclei as those located in the nucleus of solitary tract – area postrema complex in hindbrain and hypothalamus in the forebrain to control energy balance and body weight (Wilson and Enriori, 2015).

Increasing some neuropeptides will lead to increased production of anorexogenic (appetite reducing)hormones as leptin, insulin, amylin, gastric inhibitory polypeptide (GIP), Glucagon like peptide 1 (GLP - 1), Pancreatic polypeptide (PP) and Peptide Y (PY) produced in the small intestine, pancreas, and adipocytes to control energy homeostasis (Boggio, et. al., 2007). On the other hand, orexigenic hormones (appetite stimulating) as ghrelin when stimulated increase energy intake and decrease energy expenditure (Wilson and Enriori, 2015).

Ghrelin is apolypeptide hormone mainly produced by gastric enteroendocrine cells (oxyntic cells) and activated to acylated ghrelin before secretion to the blood stream by the enzyme ghrelin O acyltransferase (GOAT) in gastric mucosa. It is a potent appetite stimulator in addition to anti-inflammatory effect and aregulatory effect on gastrointestinal motility and acid secretion (Jeffery, et. al., 2013).

H. pylori is a gram negative bacterium that infects the stomach causing chronic gastritis. Although some infected individuals are symptomatic by dyspeptic symptoms, H. pylori is a major cause of peptic ulcer disease and is classified by World Health Organization as acarcinogen for gastric malignancies (Weiss, 2015).

However asubstantial proportion of infected individuals are asymptomatic (Boyanova, et. al., 2011).

Previous studies reported that gastric colonization with H. pylori has a significant effect on serum leptin level (Roper, et. al., 2008) and on gastric and plasma level of ghrelin (Isomoto, et. al., 2005). In this study, we investigated the impact of H. pylori eradication on serum leptin and plasma ghrelin in a cohort of Egyptian adult patients and impact of H. pylori eradication on BMI of these patients.

Our results showed that 35% of the forty recruited participants were males and 65% were females (Table 1) with only 32 patients responded to H. pylori eradication regimen (80%) and 8 patients had failed eradication (20%) .

There was non significant correlation between age, and sex of the included patients with H. pylori eradication. Twenty two of the eradicated H. pylori patients were females and 10 only were males. This hetrogenous constitution of the studied

population has its impact on our results and relation to results of previous studies. The important finding in our study is that there was a significant increase in plasma ghrelin level after H. pylori eradication (12 weeks after eradication) (Table 6, 17), while there was non significant difference regarding serum leptin levels before and after eradication. (Table 7, 18).

Our results agreeing with many previous studies ,regarding plasma ghrelin levels as Oswa, et al, 2006, Gunji, et al, 2008, and Kawashima, et al, 2009.

Boltin and Niv (2012) reported that weight gain following H. pylori eradication is partially explained by increased gastric expression of ghrelin and plasma ghrelin level after H. pylori eradication. However there is a considerable controversy between the results of different studies regarding the effect of H. pylori eradication on plasma ghrelin level. Nweneka and Prentice (2011) reported that seventeen out of 27 papers reported that H. pylori infection reduced plasma ghrelin levels while 10 papers reported no difference.

A little number of papers reported increased ghrelin levels after eradication of H. pylori infection while a majority of papers reported no difference. It seems that the relation between H. pylori infection and plasma ghrelin level is complex one and many factors may have a role, as H. pylori strain, severity of

gastritis and gastric atrophy, long duration of infection and follow up after H. pylori eradication.

Poulozi et al, 2013 reported that H. pylori infection directly act on mechanisms controlling ghrelin production by oxyntic cells through release of cytotoxins, lipopolysaccharides and other noxious agents and this is prominent with long duration of infection. If plasma ghrelin levels are followed up shortly after eradication of H. pylori there may be little or no difference but significantly increased levels are detected after longer duration (6 months) from eradication (Isomoto, et. al., 2005, and Lutz, et al 2006).

Chuang et al 2009, reported that males had lower plasma ghrelin levels than females when infected by H. pylori and in males only this reduction in plasma ghrelin is related to severity of gastritis .In our study, the relatively small number is a limitation to evaluate sex difference regarding ghrelin level in males and females.

Our study was conducted in adult participants but our results are agreement with results reported in children that showed increased plasma and gastric ghrelin levels in children with dyspepsia after H. pylori eradication (Deng, et. al, 2012). Another study by Yang et al 2012 showed that eradication of H. pylori increased children growth and serum acylated ghrelin

levels. Serum leptin levels did not differ significantly before and after H. pylori eradication (p = 0.38) (Table 7, 18). This is consistent with results of previous studies as Azuma, et al, 2001 and Jun, et al 2007.

However other studies reported increased leptin levels after eradication of H. pylori infection as Azquez et al 2008 and Plonka et al 2006. Vitale et al 2011, reported that H. pylori infection can reduce plasma ghrelin level and increase leptin and gastrin levels thus affecting appetite and promoting occurrence of dyspeptic symptoms. These contradictory results regarding serum leptin level and its affection by H. pylori infection can be explained by the fact that there is another major source of leptin –the adipose tissue cells-is not affected by H. pylori gastritis, so leptin is not largely affected by H. pylori eradication.

In addition some differences in follow up period after eradication and demographic characteristics of different studies can explain these contradictory results. Another important finding in our study, is the significantly increased in BMI 12 weeks after H. pylori eradication (Table 19).

This may be attributed to improved appetite and ameliorated dyspeptic symptoms as bloating and early satiation likely due to increased ghrelin level or increased acylated ghrelin which is the metabolic active form of ghrelin from the inhibitory effect of H.

pylori infection on the gastric enzyme ghrelin O acyltransferase (GOAT) (Jeffery, et. al., 2013).

Reduced ghrelin level is associated with many symptoms of functional dyspepsia and cachexia (Cheung, et al 2013), and promising trials are going on for administration of ghrelin or ghrelin receptor agonists in gastroparesis, cachexia, and cancer. Astonishing enough, is that improved BMI was observed in all included patients in this study both with successful eradication and failed eradication of H. pylori infection.

This may explained by the possibility that H. pylori treatment may help to reduce the effect of other gastric inhibitory and anorexogenic hormones as GIP and GLP-1 even in those with failed eradication and the symptomic improvement of dyspeptic symptoms as bloating and early satiation.

Boltin and Niv 2012, reported that weight gain following H. pylori eradication is poorly understood phenomenon and probably results from interacting between multiple factors including plasma and gastric ghrelin levels.

H. pylori eradication in our study population were not significantly related to any of laboratory parameters including Hemoglobin (Table 10), Serum creatinine (Table 11), Serum ALT (Table 12), Serum AST (Table 13), Serum Bilirubin (Table

14), INR (Table 15), Serum Albumin (Table 16), or BMI before treatment (Table 9).

This means that other clinical factors are more important determinents to response to H. pylori eradication as local resistance pattern to antibiotics used in eradication regimen and the patient compliance to continue the eradication regimen inspite of side effects. To sum, H. pylori eradication is necessary in symptomatic patients and this has many benefits regarding gastrointestinal and extra – gastrointestinal aspects. Improved ghrelin level (gastric and plasma) is substantial benefit that related to improved appetite and weight gain in the treated dyspeptic patients and particularly in patients with protein – energy wasted status as those with chronic renal failure on haemodialysis (Sugimoto and Yasuda 2011).

N	Sex	Age	Weigh t (k.g)	Height (cm)	BMI	S.Ghrelin Before	S.Ghrelin After	S.Leptin Before	S.Leptin After	H.P.Ag After	Hb%(g/dl)	S.Creat	ALT(mg/dl)	AST(mg/dl)	Total bilirubin	INR%	Albumin (mg/dl)
1	2	19	72	1.59	28	5500	2700	650	800	0	12.5	0.8	13	20	0.5	1.1	4
2	2	35	66	1.65	24	1000	3000	650	600	0	12	1	23	20	0.9	1	3.6
3	1	25	70	1.65	26	200	1000	3700	450	0	14.1	0.9	21	25	0.6	1	4.2
4	2	44	79	1.67	28	800	6000	30000	8000	0	13	0.7	15	18	0.8	1	3.5
5	1	43	80	1.69	28	6000	6000	1300	20000	0	13.5	1.1	20	23	0.8	1.1	3.8
6	1	23	62	1.66	22	5500	5000	13000	40000	1	12.5	0.5	12	16	0.7	1	4.3
7	2	32	70	1.64	26	3000	4500	18000	24000	0	12	0.7	19	21	0.6	0.9	3.7
8	1	34	77	1.7	27	750	2500	20000	12000	0	13	1	22	21	0.9	1.1	3.6
9	2	28	67	1.59	27	800	550	2300	30000	0	12.1	0.8	25	19	0.8	1	3.5
10	1	20	65	1.65	24	4000	800	550	2200	0	13.3	0.6	16	18	0.5	0.8	3.7
11	2	50	75	1.68	27	1000	750	220	7000	0	12.4	0.9	21	22	0.8	0.9	3.9
12	1	43	75	1.67	27	900	800	14000	1300	0	13.2	1.1	21	24	0.7	1	4.1
13	2	42	70	1.6	27	800	4500	14000	7000	0	12.6	1	12	13	0.6	1.1	4.3
14	2	35	70	1.65	26	750	5500	13000	10000	0	13	0.5	26	23	0.9	1	3.8
15	2	35	68	1.6	27	3500	5500	30000	1800	0	12	0.9	14	15	0.7	0.8	3.6
16	2	53	72	1.59	28	4500	4000	2300	4000	1	12.4	1.1	18	16	0.8	1	3.5
17	2	34	68	1.58	27	3500	4000	2800	40000	0	13	0.8	12	14	0.6	0.9	3.7
18	2	19	60	1.52	26	3500	6000	3600	1600	1	12.5	0.6	17	18	0.7	1	4.2
19	1	39	81	1.72	27	6000	3500	600	2800	1	14	1.1	25	21	0.9	1	3.6
20	2	37	54.5	1.56	22	5500	4500	550	2800	0	12.1	0.9	21	12	0.8	1	4.1

PATIENTS AND METHODS

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PATIENTS:

Forty (40) patients were above 18 years, and all patients were complaining of dyspepsia (epigastric pain and heart burn) more than four weeks

Exclusion criteria:

1. Patients on proton pump inhibitor therapy for more than five weeks.
2. Patients with alcoholic gastritis.
3. Patients with intra abdominal malignancies as lymphomas.
4. Chronic Liver disease patients as chronic viral hepatitis and cirrhosis.
5. Gastric carcinoma.
6. Patients on corticosteroid or NSAIDS medication.

Patients were selected from patients of Gastrointestinal unit, department of internal medicine, Ain Shams University Hospitals and out patient clinic.

Type of study: Cohort cross sectional prospective study.

All Patients were subjected to the following:

(1) Thorough History taking.

(2) Full relevant physical examination including abdominal

examination for organomegaly, Abdominal tenderness or masses.

- Body weight and height are determined for all patients to calculate body mass index (BMI) before eradication and 12 weeks after eradication.

(3) Laboratory investigations were included:

- Complete Blood Count (CBC).
- Liver function tests [Alanine aminotransferase (ALT), A spartate aminotransferase (AST), Serum total bilirubin and serum albumin].
- Kidney function tests (creatinine).
- Fasting blood sugar (FBS), and two hours post Prandial blood sugar (PPS).
- H-pylori status determination:

a. H. pylori was assessed using serologic method, serum samples were examined by ELISA for IgG antibodies to H-pylori whole cell and H-pylori stool antigens in stool, with results expressed as OD ratio relative to laboratory standards.

b. Subject were considered H. pylori positive if positive by H- pylori stool antigen (Hangzhou Clongene BioTech, Co, Ltd. Sheet Paper).

c. This H-pylori antigen in stool for diagnosis and to confirm eradication 4 weeks after end of the therapy.

- Pre-meal (fasting 12 hours) Serum ghrelin and serum leptin levels before eradication and 12 weeks after eradication.

A. Pre-meal Serum leptin (Human Leptin ELISA Kit):

1) Sample Preparation and Storage:

Store samples to be assayed within 24 hours at 2 - 8º C. For long-term storage, aliquot and freeze samples at -20º C Avoid repeated freeze-thaw cycles.

Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20º C.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min. at 1500 x g within 30 minute of collection. Assay immediately or aliquot and store samples at 20º C.

2) Sample Dilution Guideline

The user needs to estimate the concentration of the targe protein in the sample and select a proper dilution factor so that the

diluted target protein concentration falls near the middle of the linear regime in the standard curve.

3) Assay Procedure of leptin **(Human Leptin ELISA Kit)*:***

The ABC working solution and TMB color developing agent must be kept warm at 37ºC for 30 minute before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard Leptin detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of Leptin amount in samples:

1. Aliquot 0.l ml per well of the 4000 pg/m1, 2000 pg/m1, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml human Leptin standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1m| of each properly diluted sample of human cell culture supernates, serum or plasma (heparin, EDTA) to each empty well. It is recommended that each human Leptin standard solution and each sample be measured in duplicate.

2. Seal the plate with the cover and incubate at 37ºC for 90 min.

3. Remove the cover, discard plate content, and blot the plate onto

paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

4. Add 0.1ml of biotinylated anti-human Leptin antibody working solution into each well and incubate the plate at 37º C for 60 min.

5. Wash plate 3 times with 0.01 M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37º C for 30 min.

7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

8. Add 90ul of prepared TMB color developing agent into each well and incubate plate at 37º C in dark for 15-20 min.

9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.